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Memorandum

Date: April 25, 2003

To: The file, BLA STN 125058 (DCC83240)`

From: Howard Anderson, Ph. D.
Product Reviewer
Division of Therapeutic Proteins
Office of Therapeutic Research and Review
FDA

Through: Elizabeth Shores, Ph.D., Chief Laboratory of Immunology
Amy Rosenberg, MD, Director, Division of Therapeutic Proteins

Sponsor: BioMarin Pharmaceuticals

Product: alpha-L-iduronidase (Aldrazyme)

Subject: Review of Assays to evaluate immunogenicity of Aldurazyme for the treatment of patients with MPSI

I. Introduction

This review is an examination of assays used to evaluate potential immune responses to alpha-L-iduronidase (rhIDU, Aldurazyme). This therapeutic protein has been evaluated in patients with mucopolysaccharide storage disease category I (MPS I, Hurler's syndrome) in a phase I/II (n=10) and a phase III (n=45) clinical trial. Hurler's syndrome is a rare genetic disease that affects approximately 3000 people worldwide. Hurler's syndrome patients are genetically deficient in the lysosomal enzyme alpha L iduronidase which functions in catalyzing the hydrolysis of the terminal alpha-L-iduronic acid residue from dermatan sulfate and heparan sulfate. This disease has a broad spectrum of clinical manifestations. Patients evaluated in these clinical trials have approximately 1.5 % of normal iduronidase activity. Patients with reduced or absent alpha-L-iduronic activity accumulate glycosaminoglycans (GAG) throughout all tissues of the body which results in organ dysfunction and often death by the second decade of life.

rhIDU is a soluble monomeric glycoprotein (83 kDa) produced in CHO cells. It is administered intravenously weekly for enzyme replacement therapy to Hurler's syndrome patients. The lysosomal enzyme is rapidly cleared from the blood ($t_{1/2}$=4 hrs) and it is internalized into cells and targeted to lysosomes via the mannose 6 phosphate receptor. This protein appears to be highly immunogenic and IgG antibody titers are detected in all patients injected with the protein. -------- ----

------analysis (phase II) and ---------- (phase III) indicate that specific IgG antibodies are developed to rhIDU as well as to a CHO host cell protein contaminant. Patients develop antibodies to rhIDU with a mean time to sero-conversion of 52 days. Patients administered rhIDU develop hypersensitivity responses which require that they be treated with antipyretics and antihistamines prior to administration. To better understand hypersensitivity reactions associated with Aldurazyme treatment the sponsor has developed an ELISA assay for the detection of anti-rhIDU IgE antibodies in patient's serum. Serum was collected from patients who demonstrated injection associated hypersensitivity reactions (n=3). In addition, complement-activation testing was also performed on these sera. The results of testing of serum from the 3 patients who demonstrated injection associated hypersensitivity reactions failed to detect specific anti-rhIDU IgE antibodies and did not detect complement activation. However, one patient in the phase III study demonstrated a serious adverse event (hypersensitivity reaction) that was associated with rhIDU treatment. Testing of the patient's serum revealed the presence of anti-rhIDU specific IgE antibodies.

II Screening assay- IgG-ELISA

a. Description of protocol

Phase III (n=45)

IgG testing was performed on serum taken from all patients just prior to the administration of rhIDU and at weeks 4, 8, 12, 16, 20, and 26. rhIDU FBDS (formulated bulk drug substance) is ----------

Phase II (n=10)

Essentially the same ELISA assay was used to evaluate IgG responses in the phase II trial as was used in the phase III trial. The one major difference is that ---------- Data are provided to demonstrate that the two assays used in the phase I/II and phase III study are comparable.

Appropriateness of reagents

All reagents used in the IgG ELISA assay are scientifically valid. It should be noted that the detection antibody reacts with human IgG whole molecule and is therefore likely to detect all Ig isotypes and IgG subclasses.

Method of reporting

The results of ELISA testing are reported as OD----/ul of serum. This value is calculated by multiplying the dilution factor by the OD---- value (mean of n=---) and dividing this number by the volume of serum assayed ---------- Multiple serum dilutions are assayed to ensure that values are within the linear range of the assay. It should be noted that OD----- values are determined by subtracting background values (blank, OD-----------). The sponsor states that the linear range of the assay has been determined to be between three times base line and less that 1.5. Data are provided to support this conclusion.

b. Definition of Cutoff Value
Method
[

] There is adequate sample testing (>30 samples) to support this value.

Confidence Intervals
CV values of replicate samples must be less than or equal to -----. Data are provided to support this value.

c. Reproducibility
Intra-Assay Precision [

]

Inter-assay precision [

]

d. Sensitivity of the Assay
Method of reporting
A positive response is defined as OD -----/µl of serum with a value [

]

How determined
Administration of rhIDU results in the generation of specific IgG titers in most individuals (20/22). The anti-rhIDU IgG ELISA has been validated with serum derived from patients injected with rhIDU.
[

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[

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d. Specificity
Method
[

]

Implications
The above methods indicate that OD---- values reflect the presence of anti-rhIUD IgG antibodies and the assay results are not the result of antibody reacting with something other than Aldrazyme drug product. It should be noted that competition experiments using Aldrazyme have not been performed.

Results
The IgG screening ELISA assay has the ability to specifically detect anti-rhIDU IgG antibodies since detection is dependent on antigen and serum obtained from individuals injected with rhIDU. In the phase III study 20 of the 22 patients developed IgG titers with an average time to conversion equal to 53 days

e. Supporting Data- ---------- Assay for patient IgG-specific immune responses to rhIDU

[

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III. Other Assays to Measure Immune Responses

a. IgE ELISA- Description of protocol
IgE levels were measured in samples taken from patients enrolled in the phase III clinical trial. Recombinant human ---- (Genzyme Clinical supply unit) is [

]

b. Appropriateness of reagents

All reagents are scientifically valid. The positive control antiserum consists of a ------- anti rhIDU IgG preparation that was generated by injecting --------- with rhIDU in ----------. ---------- ----------

c. Method of reporting
[

]

d. Definition of Cut off value
[ ]

e. Repeatability
[

]

f. Sensitivity of the Assay
[

]

g. Specificity
[

]

h. Other Validation Results
The sponsor has submitted data to indicate that the assay is consistent across the plate. The ---------- positive control serum is not sensitive to ----------. In addition positive serum is not effected by EDTA or Heparin.

i. Results- of IgE
Testing was performed only on patients who experienced severe infusion associated adverse events (n=3). Sponsor failed to detect anti-rhIDU IgE antibodies in these serum. During the phase III open label extension an individual developed a anaphylactic reaction that was associated with Aldrazyme administration. Testing of the patient's serum detected anti-Aldrazyme IgE.

j. Summary of Results

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IV Neutralizing Assay

1. Description of Assay

Serum samples were obtained form patients at baseline and week 26 (phase III double blind study) to determine if anti-Iduronidase antibodies inhibit enzyme activity. The neutralizing assay was performed [

]

3. Appropriateness of reagents

All reagents are scientifically appropriate. ---------- serum is obtained from ---------- ---------- anti-rhIDU antibody (----------) is used for a positive control. This antibody was made by BioMarin and is ----------. A negative control ----------antibody, ----- is also ----------. Serum from a patient in the phase III clinical trial demonstrates neutralizing activity. The serum inhibits enzyme activity by [

]

4. Definition of Cut Off Value

[

]

5. Sensitivity of the Assay

The assay is able to specifically detect enzyme inhibition of ---------- serum). This is defined by determining that normal serum demonstrates a ------- inhibitory effect on enzyme activity compared to enzyme activity observed in buffer only.

6. Specificity of the Assay

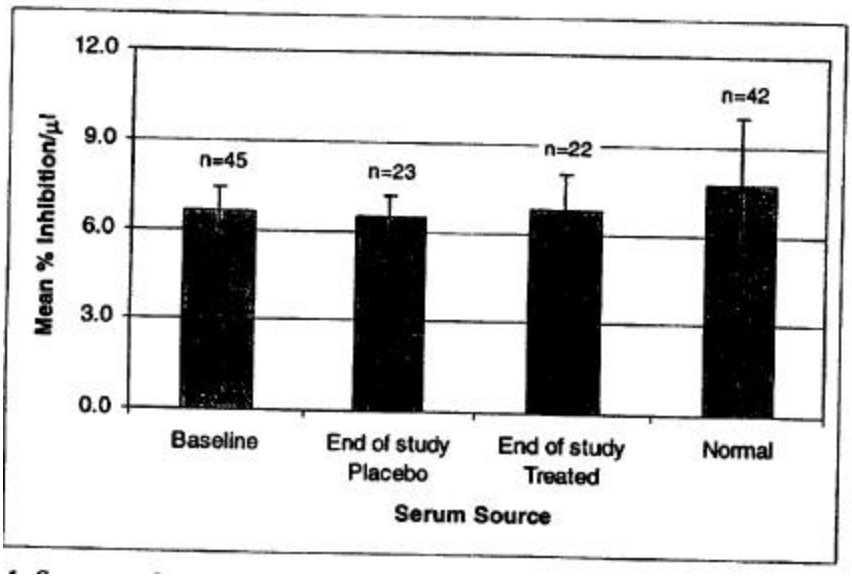
The percentage of inhibition for control --Ab ranged from ---------- inhibition across all dilutions assayed. The mean % inhibition from serum from normal ---------- (range ---------- % inhibition/ul)

7. Reproducibility of the Assay

The assay has been qualified and data are provided to support the following; rhIDU activity measured in serum is linear and that activity is a direct result of rhIDU enzymatic activity (Accuracy). Data submitted indicate that intra-assay precision is ------ (same plate) and inter-assay precision CV vales were ---------. For inhibition with positive control antibody (---------- ----------) CV value was ------- and the CV value for positive control serum was also -------

8. Results

Group	Patient Designation	Result (% Inhibition/µl)		Difference End of study - Baseline (Δ % Inhibition/µl)
		Baseline	End of Study	
Placebo	01-02-02	6.5	7.3	0.8
	01-03-03	<min quant	<min quant	0.0
	01-05-05	<min quant	<min quant	0.0
	01-07-07	<min quant	<min quant	0.0
	01-09-09	<min quant	<min quant	0.0
	01-13-12	<min quant	6.4	0.4
	05-08-07	<min quant	<min quant	0.0
	03-01-01	8.5	6.7	-1.8
	03-02-03	6.3	<min quant	-0.3
	03-03-09	<min quant	<min quant	0.0
	03-05-02	6.8	6.4	-0.4
	03-07-05	<min quant	6.3	0.3
	04-03-03	7.0	6.6	-0.4
	05-06-05	<min quant	8.6	2.6
	04-02-02	<min quant	<min quant	0.0
	06-02-02	6.9	7.5	0.6
	04-05-05	7.6	6.4	-1.2
	06-03-03	<min quant	<min quant	0.0
	05-02-02	8.5	7.4	-1.1
	06-06-07	<min quant	<min quant	0.0
	05-04-03	7.5	7.2	-0.3
	06-07-05	6.4	<min quant	-0.4
	06-08-09	<min quant	<min quant	0.0
	Placebo Means:	6.5	6.5	-0.1
Treated	01-01-01	<min quant	<min quant	0.0
	01-04-04	7.7	<min quant	-1.7
	01-06-06	<min quant	6.3	0.3
	01-08-08	<min quant	<min quant	0.0
	01-10-10	7.9	6.1	-1.8
	01-12-11	6.9	<min quant	-0.9
	06-01-01	9.1	<min quant	-3.1
	06-04-04	<min quant	<min quant	0.0
	03-04-04	<min quant	<min quant	0.0
	03-06-08	<min quant	7.2	1.2
	03-08-06	6.1	6.3	0.2
	03-09-10	<min quant	9.4	3.4
	06-10-10	6.7	<min quant	-0.7
	05-01-01	7.7	6.4	-1.3
	06-09-08	6.5	7.1	0.6
	05-05-04	7.1	9.8	2.7
	06-11-11	6.9	7.7	0.8
	04-06-06	<min quant	9.5	3.5
	04-01-01	<min quant	<min quant	0.0
	05-07-06	7.2	7.1	-0.1
	04-04-04	<min quant	<min quant	0.0
	06-05-06	6.8	6.4	-0.4
	Treated Means:	6.7	6.8	0.1



V Effect of Antibodies on pK

Figure 5: Individual Patient $t_{1/2}$ after Infusions 1, 12, and 26: Study No. ALID-003-99

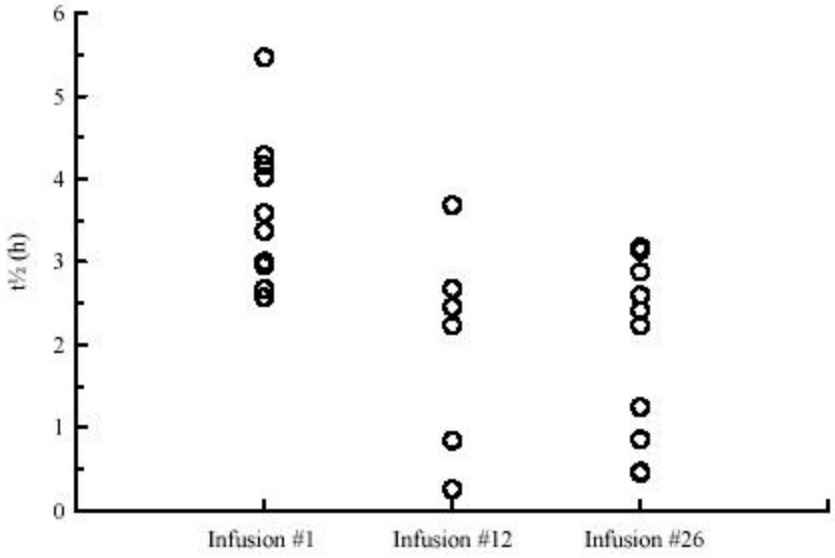
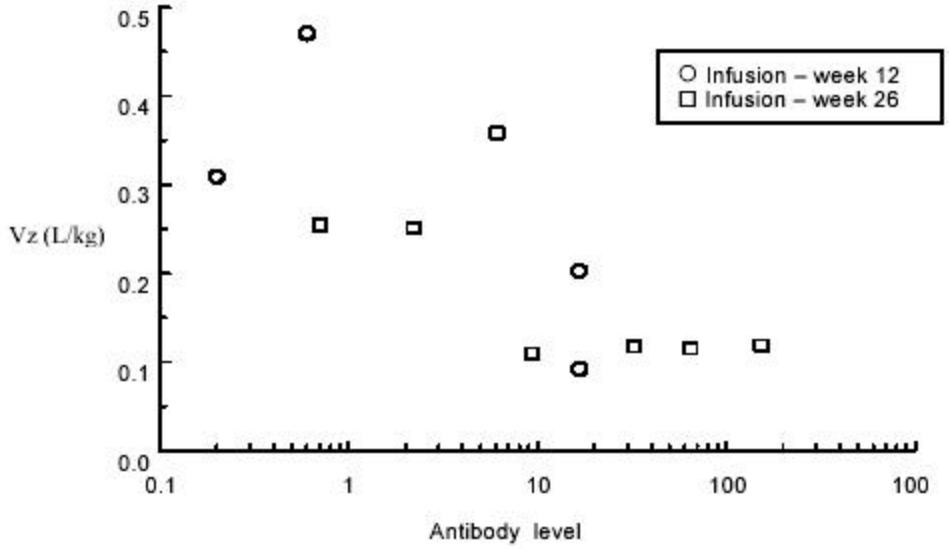


Figure 6: Relationship between V_z and Antibody Level (ELISA) after the 12th and 26th Infusions: Study No. ALID-003-99



VI FDA Review of Immunogenicity Assays

Overall the IgG screening ELISA assay and the confirmatory RIP Assay are adequate to demonstrate that patients develop IgG antibodies that react with Aldurazyme. However, the assays are qualitative in nature and the sponsor needs to quantify specific antibody levels. This is important as patients will remain on Aldurazyme therapy for extended periods of time and it is important to determine if antibody titers change as a function of time (e.g. do patients become tolerant to Aldurazyme). It should be noted in the Endocrinologic and Metabolic Drugs Advisor Committee (1/15/03) the committee recommended that serum from individuals injected with Aldrazyme should be taken and stored for an unspecified period of time so that studies can be performed to better understand immune responses to Aldrazyme.

The assay that the sponsor has developed to determine if anti-Aldurazyme antibodies neutralize enzyme activity lacks a sensitive positive control. For validation of the assay this should be performed by the sponsor. The sponsor states that normal serum inhibits enzyme activity by 15 % and an anti-Aldurazyme monoclonal antibody inhibits enzyme activity by 30 %. In addition, testing of one patient's serum indicates that 4 weeks after Aldurazyme therapy, the patient's serum inhibited enzyme activity by approximately 25 %, subsequent testing of the serum taken from the patient at weeks 5 and 6 after treatment indicate that serum lost inhibitory activity. Some of the patient's serum appears to weekly neutralize enzyme activity (e.g. 20 % inhibition). Thus, in the absence of a good positive control it is unclear to what extent, if any, that IgG titers detected in patient's serum neutralize enzyme activity. The sponsor claims in the proposed package insert that [

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It is premature to make this claim and further testing of patients serum should be conducted. It is also unclear at this time if IgG titers affect the pharmachokinetics and distribution of Aldurazyme. The sponsor provides limited data that anti-Aldurazyme may effect clearance of the enzyme (see section E). [

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Post-Approval Immunogenicity Commitments:

1) [

] To determine how antibody levels may affect drug efficacy with recommend that in addition to obtaining serum urine samples be obtained from the same individuals for quantification of GAG level samples to determine if immune response affects drug efficacy.

[

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The sponsor was issued a CR letter on 1/28/03. As part of the letter the FDA requested post-marketing commitments to further study patient's immune responses to Aldurazyme. For a review of these commitments please see Dr. Anderson's Review of Sponsor's response to the CR letter.